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Soil Carbon, Nitrogen, and Ergot Alkaloids with Short- and Long-Term Exposure to Endophyte-Infected and Endophyte-Free Tall Fescue

A. J. Franzluebbers* and N. S. Hill

ABSTRACT

Tall fescue (*Festuca arundinacea* Schreb.) is an important cool-season perennial forage naturally infected with an endophyte, *Neotyphodium coenophialum* Glenn, Bacon, & Hanlin, which produces ergot alkaloids. We conducted a controlled incubation study to determine the fate of C, N, and ergot alkaloids in tall fescue leaf tissue added to soil. The experimental setup was a factorial combination of endophyte-free (E−) and endophyte-infected (E+) leaf tissue (short term) incubated in soil exposed to 10 yr of E− and E+ tall fescue pasture (long term). Soil history of E+ compared with E− reduced C mineralization per unit of soil organic carbon (52 vs. 55 mg g^{-1} SOC) and the fraction of inorganic N as nitrate (0.68 vs. 0.72 g g^{-1}), but increased ergot alkaloid concentrations in soil sediment (<1 mm; 28 vs. 12 ng g^{-1}), coarse fraction (>1 mm + remaining leaves; 5.8 vs. 2.2 ng g^{-1}), and water extract (0.27 vs. 0.22 ng g^{-1} soil). Short-term exposure of soil to E+ leaves compared with E− leaves reduced C mineralization (660 vs. 688 μg g^{-1} soil) and soil microbial biomass C (487 vs. 583 μg g^{-1} soil), but increased net N mineralization (70 vs. 59 μg g^{-1} soil), soil microbial biomass N (56 vs. 19 μg g^{-1} soil), and ergot alkaloid concentration in the coarse fraction (0.36 vs. 0.27 μg g^{-1} original leaf). Both short- and long-term exposure of soil to E+ tall fescue were affecting soil organic matter dynamics by altering biochemical transformations of C and N. Our results suggest that wild-type E+ tall fescue can alter soil organic C storage through a reduction in soil microbial activity. This research has also demonstrated the presence of ergot alkaloids in soil under E+ tall fescue.

Tall fescue is the most widely adapted cool-season perennial forage in the eastern USA (Stuedemann and Hoveland, 1988). A natural association with a fungus (*N. coenophialum*; formerly called *Acremonium coenophialum* Morgan-Jones and Gams) often results in improved persistence of tall fescue in the seasonally drought- and heat-stressed region of the southeastern USA (Hill et al., 1991; Bouton et al., 1993). Reasons for its persistence have been attributed to physiological responses that confer greater drought tolerance (Belesky et al., 1987a; West et al., 1993) and production of ergot alkaloids that may reduce forage intake by grazing animals, thereby reducing grazing pressure (Hoveland et al., 1983; Read and Camp, 1986; Hill et al., 1990). Toxic effects of E+ tall fescue on grazing animals, causing such disorders as fescue foot, fat necrosis, and fescue toxicosis, have been well documented (Stuedemann and Hoveland, 1988; Bacon and Hill, 1997).

Ecologically, the presence of *N. coenophialum* may be important in reducing a variety of insect pressures on tall fescue pastures (West et al., 1988; Clay, 1993; Siegel and Bush, 1996), as well as controlling plant diseases (Latch, 1997). A variety of alkaloids, N-containing ring structures, are produced in the tall fescue-*Neotyphodium* association (Bush et al., 1993), of which some may be more important in controlling insect and mammalian herbivory and altering plant physiological responses to stress. Pyrrolizidine alkaloids can deter insect feeding (Johnson et al., 1985). Ergot alkaloids appear to be responsible for toxic conditions in cattle (Hill et al., 1994). *Neotyphodium* also infects a variety of natural grass populations around the world, and in some cases, decreases the fitness of plant hosts due to competition for nutrients between plant host and endophyte (Faeth et al., 2004).

Endophyte associations with tall fescue have been recently manipulated, such that strains of fungus with low ergot alkaloid production have been selected and combined with improved cultivars of tall fescue (Bouton et al., 2002). A distinction can now be made between wild-type endophyte association (occurring naturally with high ergot alkaloid production) and novel endophyte association (selected fungus with little or no ergot alkaloid production).

Pastures in Georgia containing a high occurrence of E+ tall fescue were found to be associated with greater soil organic C and N concentration and lower potential soil microbial activity than pastures with low occurrence of endophyte infection (Franzluebbers et al., 1999). These data suggested that soil microbial activity might be sufficiently reduced by the presence of endophyte metabolites (i.e., various alkaloids and phenolics produced by the endophyte association), as to eventually lead to greater accumulation of soil organic C and N due to reduced decomposition of plant-derived compounds. Support for this hypothesis of reduced microbial activity with endophyte infection was demonstrated in an outdoor microcosm study in Argentina, where leaf litter from E+ Italian ryegrass (*Lolium multiflorum* Lam.) decomposed slower than E− leaf litter (Omacini et al., 2004).

We conducted a controlled incubation study to test the hypothesis that E+ tall fescue leaves would reduce C and N mineralization, as well as the agent of many biochemical transformations in soil, the microbial biomass. We tested this hypothesis by exposing soil to endophyte metabolites from fresh tall fescue leaves and from long-term pasture growth. We also tested a hypothesis that soil exposed to endophyte metabolites for many years might condition soil to overcome any reduced potential to decompose freshly added E+ tall fescue leaves. Finally, we wanted to determine the fate and

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Abbreviations: E−, endophyte free; E+, endophyte infected; SOC, soil organic carbon.

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dynamics of ergot alkaloids added in E+ tall fescue leaves during incubation in soil.

MATERIALS AND METHODS

A replicated field experiment near Watkinsville, GA (33°62′ N, 83°25′ W) on a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludult) was the source of soil and plant material for this incubation study. Two treatments (E− and E+ 'Kentucky 31' tall fescue) were grown in two 1.6-ha blocks for a total of four paddocks. Endophyte-infected paddocks (100% seed infection) were planted in autumn of 1987. Endophyte-free paddocks (0% seed infection) were planted in autumn of 1988. Seed for the E− treatment came from the same lot as the 100%-infected source, which had been incubated at ambient temperature for 1 yr to reduce endophyte viability. Endophyte infection frequency of pastures was <1% in the E− treatment and 88% in the E+ treatment at the last sampling in 1996 (Franzluebbers et al., 1999). It is possible that the occurrence of E+ plants increased thereafter in E− pastures, similar to the 1.4 ± 0.9% yr^{-1} that occurred in other nearby pastures (Franzluebbers and Stuedemann, 2005).

From each of the four paddocks, a composite soil sample (≈3.5 kg dry wt.) was collected on 25 May 1999 from 20 cores (4-cm diam.) at a depth of 3 to 12 cm (soil depth chosen to represent rooting zone, but also to avoid excessive particulate organic matter accumulation at the soil surface). Soil was brought to the laboratory, sieved through a 4.75-mm screen to remove stones and to homogenize the composite sample, and allowed to dry for 2 d with periodic mixing from an initial water concentration of 0.10 ± 0.01 g g^{-1} to a final concentration of 0.05 ± 0.03 g g^{-1}. From each of the four paddocks, a composite tall fescue leaf sample (≈200 g dry wt., 20-cm length) was collected on 27 May 1999 from random locations within the paddock. Fresh leaves were cut into ≈1-cm segments, mixed, and stored in a sealed plastic bag for 4 d at 4°C until the incubation began. Subsamples of soil and leaves were dried at 55°C for 24 h for chemical analyses (Table 1).

The experimental design was a factorial arrangement of soil history (exposed to E− and E+ tall fescue) and tall fescue leaf addition (none, E−, and E+ tall fescue) with multiple experimental units for analysis at different times of incubation (0, 1, 2, 4, 8, 16, and 32 d).

For each of the two field replicates of each soil history, 24 glass jars (1 L) were filled with 100-g dry-weight equivalents of soil (total of 96 jars). One of three leaf addition treatments was added to each of the jars (no leaves, leaves from the same paddock as the soil, or leaves from the adjacent treatment in the same block) for a total of eight jars per treatment and replicate. A total of 12 jars (three leaf treatments × two soil history treatments × two field replicates) were destructively sampled at each of seven times during a 32-d incubation period. A final set of 12 jars was evaluated separately for soil microbial biomass properties at the end of 32 d. Five grams of fresh leaf tissue were added (1.44 ± 0.09 g dry wt) and mixed with the soil. Deionized water was added to each of the incubation jars to achieve a final water concentration of 0.27 g g^{-1} or 50 to 60% water-filled pore space, considered optimum for decomposition (Franzluebbers, 1999). An alkali trap (40 mL of 0.5 *N* NaOH) was placed on a platform above soil in each jar and jars incubated at 25 ± 1°C. Alkali traps were replaced at the end of 1, 2, 4, 8, 16, and 32 d of incubation. Carbon dioxide released during incubation was determined from titration of alkali traps to a phenolphthalein endpoint following precipitation with excess $BaCl_2$ (Zibilske, 1994). A set of five jars without soil were distributed throughout the experimental setup and used as blanks for determining background CO_2.

Table 1. Soil properties as affected by long-term exposure (i.e., 10 yr) to endophyte-free (E−) and endophyte-infected (E+) tall fescue and tall fescue leaf properties added to soil (n = 2).

	E−		E+	
Property	**Mean**	**SD**	**Mean**	**SD**
	mg g^{-1}			
Soil organic C	8.5	0.2	9	0.8
Total soil N	0.44	0.02	0.46	0.05
Soil inorganic N	0.014	4	0.012	0.003
Leaf water	698	3	727	4
Leaf C	397	2	391	3
Leaf N	31	3	32	3
Leaf ergot alkaloids	0.00049	0.00003	0.00193	0.00007

At the end of 32 d of incubation, the remaining set of 12 jars were fumigated for 24 h with $CHCl_3$, aerated to remove vapors, an alkali trap added, and incubated for a further 10 d under the same conditions as before (Jenkinson and Powlson, 1976). Soil microbial biomass C was calculated from the quantity of CO_2 evolved during 10 d following fumigation divided by an efficiency factor of 0.41 (Voroney and Paul, 1984). Specific respiratory activity of soil microbial biomass was calculated from the linear rate of C mineralization between 16 and 32 d of incubation divided by soil microbial biomass C. Carbon mineralization between 16 and 32 d of incubation was expected to avoid the flush of mineralization during early stages of leaf decomposition (Franzluebbers et al., 1994b).

At each sampling event (i.e., 0, 1, 2, 4, 8, 16, and 32 d of incubation), a total of 12 jars were removed from incubation and shaken with 200 mL of deionized water for 30 min at 150 cycles min^{-1} to collect three fractions: (i) coarse sand + remaining leaves to determine ergot alkaloid concentration of undecomposed leaves, (ii) water extract to determine soluble ergot alkaloid and inorganic N concentrations, and (iii) soil sediment to determine ergot alkaloid concentration associated with soil particles. Fractions were collected according to the following. Contents of a jar were poured over a screen with 1-mm openings resting on a collection vessel. The screen allowed passage of the major portion of soil, while retaining tall fescue leaf pieces following incubation. An additional 50 mL of water were used to complete the transfer of contents from the jar. The vessel and screen were vortexed for 1 min to complete separation of material at 1-mm size. Sand and leaves remaining on the screen were washed in a stream of water for ≈15 s, transferred to a labeled container, and frozen (−20°C) (coarse sand + remaining leaves). The vessel containing water extract and soil < 1 mm was decanted into a labeled container with ≈70 mL of solution collected and frozen (water extract). The remaining solution above the soil sediment was discarded, and ≈45 g of sediment was transferred into a labeled container and frozen (soil sediment).

The water extract was thawed and a 10-mL aliquot removed with a filtered syringe for determination of inorganic N (NH_4–N + NO_2–N + NO_3–N) concentration using salicylate-nitroprusside and Cd-reduction autoanalyzer techniques (Bundy and Meisinger, 1994). The remaining solution was refrozen. This process of freezing–thawing–freezing and subsequent thawing a second time allowed clay and silt suspended in solution to precipitate. Two 15-mL aliquots of cleared solution were transferred into vials, frozen, and freeze-dried before ergot alkaloid determination.

The frozen leaf + coarse sand fraction was freeze-dried, weighed, and ground to a fine powder in a ball mill for 5 min. In addition, a subsample of frozen leaf tissue not exposed to incubation and the frozen soil sediment fraction were freeze-

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dried and ground in a ball mill before ergot alkaloid determination.

Ergot alkaloid concentration of freeze-dried fractions was determined using a competitive enzyme-linked immunosorbance assay outlined by Hill and Agee (1994). The antibody used was specific to the lysergic moiety of all ergot alkaloids, and has the capacity to reverse physiological symptoms of circulating toxins in cattle (Hill et al., 1994). Ergot alkaloids were extracted from 1.5 g of soil sediment or leaf + coarse sand fraction in a 10-mL polyethylene bottle with 8 mL of extraction buffer (1.17 g Na_2HPO_4, 0.288 g NaH_2PO_4, 8.175 g NaCl, and 0.5 mL Tween 20 [polyoxyethylenesorbitan monolaurate; Cayman Chemical Company, Ann Arbor, MI][1] L^{-1} H_2O). Ergot alkaloids in freeze-dried leaves were determined from 0.1 g in 8 mL of extraction buffer. Ergot alkaloids in the water extract were determined by resuspending the freeze-dried water in 0.5 mL of extraction buffer. Immulon IV microtiter plates (Thermo Labsystems, Franklin, MA) were coated overnight at 4°C with 188 pg of lysergol conjugated to human serum albumin, blocked for 30 min at ambient temperature with bovine serum albumin, and washed. A 1-mL subsample of alkaloid extract was transferred into a 1.5-mL disposable centrifuge tube to separate solids from liquid by centrifuging at 10 000 rpm. Lysergic acid standards (0, 1.6, 3.1, 6.3, 12.5, and 25 μg lysergic acid L^{-1} extraction buffer) were prepared, and 50 μL added to a well of a microtiter plate. Monoclonal antibody 15F3.E5 was diluted 1:80 in borate saline solution (6.19 g H_3BO_3, 9.5 g $Na_2B_4O_7{\cdot}10H_2O$ 4.39 g NaCl L^{-1} H_2O) and 50 μL added to each well containing extracted sample. The plate was incubated at 21°C for 2 h and washed. Rabbit anti-mouse antibody with alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis) was added to wells and the mixture incubated for 1 h. After washing the plates, ρ-nitrophenyl phosphate (Sigma Chemical Company, St. Louis, MO) was added, the plates incubated for 10 min, and the reaction stopped by adding 50 μL of 3 *M* NaOH. Plates were read at 405 nm using a Bio-TEK EL800 microplate reader (Winooski, VT). Ergot alkaloid concentration was determined from a calibration curve of standard lysergic acid concentration regressed on absorbance, using a cubic fit.

Data were analyzed for variance with the general linear models procedure of SAS (SAS Institute, 1990). Initially, data were analyzed for each sampling event separately. Data were then pooled across sampling events, with sampling event considered a blocking criterium, because soil properties were expected to change magnitude with time. Long-term exposure to E+ tall fescue pasture was evaluated by averaging properties across leaf addition treatments, since most soil properties did not exhibit significant interactions between soil history and leaf addition (Table 2). Differences among all means were considered significant at $P < 0.1$. A 2-pool nonlinear + linear model {cumulative C mineralization = $C_0[1 - \exp(-k \times t)]$ + BSR × t} was fitted to cumulative C mineralization for amended soils using the regression wizard of SigmaPlot v. 5.0 (SPSS, 1999), where C_0 was the size of the rapidly mineralizable C pool (μg g^{-1}), k was the nonlinear rate constant (d^{-1}), BSR was basal soil respiration (μg g^{-1} d^{-1}), and t was time (d). Unamended soils did not exhibit an initial flush of CO_2, and therefore, a linear model (cumulative C mineralization = BSR × t) was fitted to cumulation C mineralization. An exponential decay model [$A_t = A_0 + A_1 \times \exp(-k \times t)$] was fitted to ergot alkaloid concentration during a given time of incubation (A_t) using the regression wizard of SigmaPlot, where A_0 was the basal concentration or sill (μg $vessel^{-1}$), A_1 was the size of the pool that undergoes decomposition (μg $vessel^{-1}$), k was the nonlinear rate constant (d^{-1}), and t was time (d).

[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

RESULTS AND DISCUSSION

Active Soil Carbon and Nitrogen in Response to Short-Term Exposure of Endophyte-Infected Tall Fescue Leaves

Addition of tall fescue leaves to soil had a dramatic positive effect on CO_2 evolution (Fig. 1) and inorganic N accumulation (Fig. 2) as expected, because of the high concentration of these nutrients in leaf tissue (Table 1). A relatively small, but significant, modification of this response occurred, in which addition to soil of E+ compared with E− tall fescue leaves reduced C mineralization in both soil histories (Fig. 1). The 2-pool (nonlinear + linear) regressions fitted the experimental data from leaf-amended soils very well ($r^2 = 0.99$ in all cases). Across soil histories, there was a 4 + 1% larger rapidly mineralizable pool of C (C_0) from added E+ leaves than from E− leaves (Table 3). As a percentage of the total amount of C added with leaves, C_0 was 21% for E− and 25% for E+ leaf additions. However, the positive effect of E+ leaf addition on C_0 was counteracted with lower rate constants for both the nonlinear (i.e., k) and linear pools (i.e., BSR). To achieve 95% mineralization of C_0, 25.8 and 28.6 d would have been required for E− and E+ tall fescue leaf additions, respectively. Therefore, at least

Table 2. Analysis of variance in soil properties (expressed as the probability of achieving a greater *F* value, Pr > *F*) as affected by incubation time (T), soil history (S), and leaf addition (L).†

Source of variation	df	CMIN	NMIN	C/N MIN	NO_3/N	Ergot alkaloids in Soil sediment	Ergot alkaloids in Coarse fraction	Ergot alkaloids in Water	SMBC	SMBN	C/N SMB
		Pr > *F*									
T	6	<0.001	<0.001	<0.001	<0.001	0.28	0.002	<0.001	na	na	na
S	1	0.61	0.69	0.64	0.02	0.01	<0.001	0.008	0.30	0.37	0.87
T × S	6	0.99	0.54	0.86	0.59	0.26	0.09	0.80	na	na	na
L	2	<0.001	<0.001	<0.001	<0.001	0.30	0.001	<0.001	0.002	0.02	0.07
T × L	12	<0.001	<0.001	0.02	<0.001	0.16	0.29	0.007	na	na	na
S × L	2	0.96	0.80	0.97	0.51	0.22	0.74	0.24	0.18	0.22	0.34
T × S × L	12	0.99	0.80	0.99	0.93	0.10	0.24	0.47	na	na	na

† CMIN, potential C mineralization; NMIN, potential N mineralization; C/N MIN, mineralizable C/N ratio; NO_3/N, fraction of inorganic N as nitrate; SMBC, soil microbial biomass carbon; SMBN, soil microbial biomass nitrogen; C/N SMB, soil microbial biomass C/N ratio. Ergot alkaloids were determined in soil sediment (<1 mm), coarse fraction (>1 mm, coarse sand + leaves), and water extract. na is not applicable. SMBC, SMBN, and C/N SMB were determined only at the end of 32 d of incubation.

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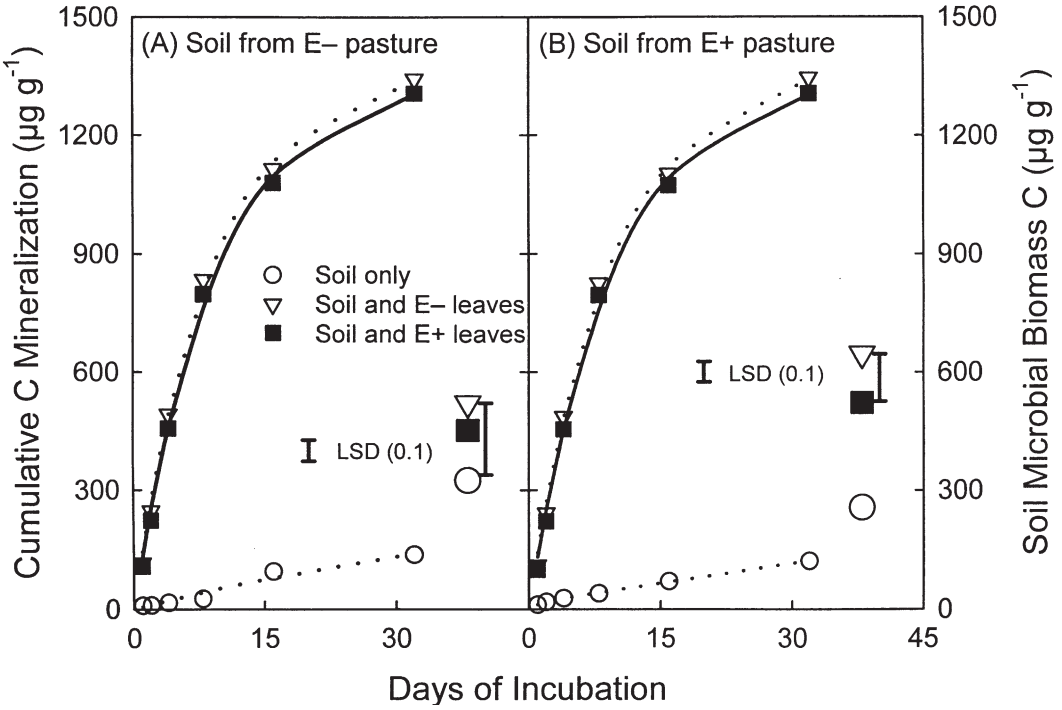

Fig. 1. Cumulative C mineralization during 32 d of incubation and soil microbial biomass C at the end of 32 d of incubation as affected by addition of endophyte-free (E−) and endophyte-infected (E+) tall fescue leaf tissue in soil exposed to long-term history of E− and E+ tall fescue.

during the short-term exposure period of 32 d in this study, C mineralization from E+ tall fescue leaves was slower than from E− tall fescue leaves. Should the calculated linear rate of C mineralization (2.8 vs. 0.6 µg g^{-1} d^{-1} from E− and E+ leaves, respectively) following the initial rapid flush continue beyond the time frame of this study, there would be a significant suppression of C mineralization from added E+ leaves. In fact, even during 32 d of incubation, cumulative C mineralization was lower (P = 0.003) when exposed to E+ than E− tall fescue leaves when averaged across sampling events and soil histories (Table 4).

Soil microbial biomass C at the end of 32 d of incubation was lower (P = 0.08) when exposed to E+ than

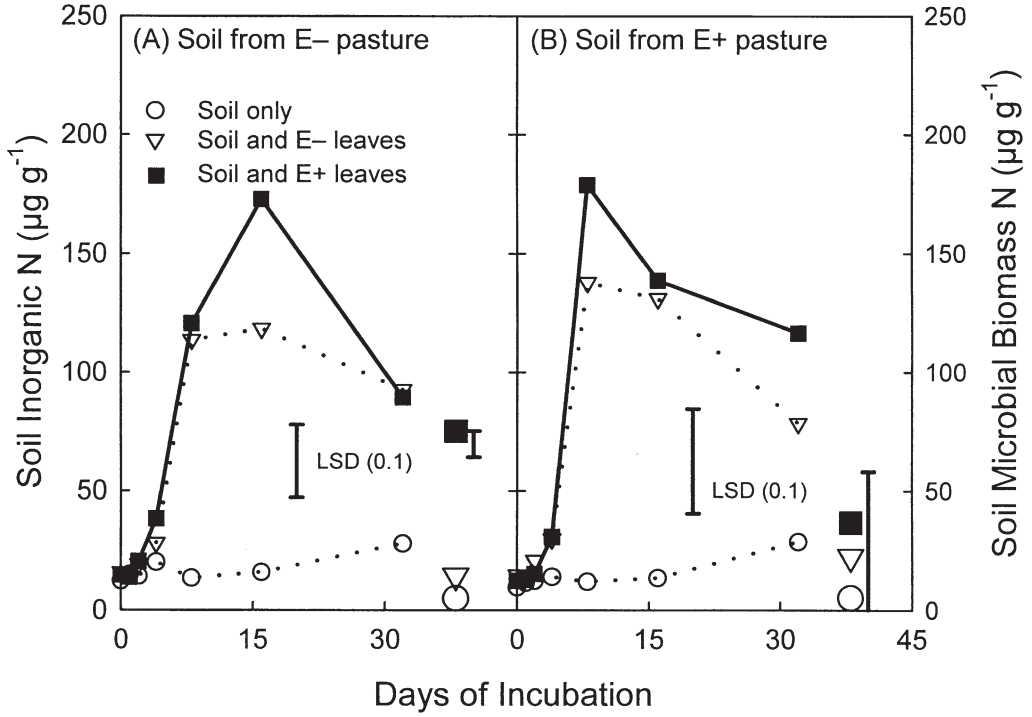

Fig. 2. Soil inorganic N accumulation during 32 d of incubation and soil microbial biomass N at the end of 32 d of incubation as affected by addition of endophyte-free (E−) and endophyte-infected (E+) tall fescue leaf tissue in soil exposed to long-term history of E− and E+ tall fescue.

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Table 3. Regression parameters describing cumulative C mineralization as affected by long-term history (i.e., 10 yr) of soil exposed to tall fescue and short-term addition of tall fescue leaves (E− is endophyte-free, E+ is endophyte infected) during 32 d of incubation. Regression parameters describe cumulative C mineralization according to the equation, $C_0[1 - \exp(-k \times t)] + BSR \times t$, where C_0 is the size of the rapidly mineralizable C pool (μg g^{-1}), k is the nonlinear rate constant (d^{-1}), BSR is basal soil respiration (μg g^{-1} d^{-1}), and t is time (d). Probability of achieving a greater F value ($Pr > F$) indicates the likelihood of similarity in short-term leaf addition (E− vs. E+) treatment populations.

	C_0			k			BSR		
Soil history	E−	$Pr > F$	E+	E−	$Pr > F$	E+	E−	$Pr > F$	E+
E−	1299	0.001	1354	0.116	0.04	0.104	2.3	0.08	5×10^{-9}
E+	1271	0.070	1313	0.116	0.28	0.106	3.3	0.11	1.2
Mean	1285	0.002	1333	0.116	0.01	0.105	2.8	0.002	0.6

E− tall fescue leaves when averaged across soil histories (Table 4), although with a larger depression in soil from E+ pasture (Fig. 1). Specific respiratory activity of soil microbial biomass was not affected ($P = 0.15$) by exposure to E+ compared with E− tall fescue leaves, but both were higher than unamended soil (Table 4). The suppressive effect on soil microbial biomass C with short-term exposure to E+ tall fescue leaves was consistent with C mineralization, suggesting that reduced C mineralization with E+ was not a result of an engorged microbial biomass pool that could have delayed complete mineralization of organic C compounds.

In contrast to C pools, net N mineralization and soil microbial biomass N were higher ($P = 0.08$ and 0.02, respectively) when exposed to E+ than E− tall fescue leaves (Fig. 2, Table 4). In response to these contrasting results, mineralizable C/N ($P = 0.05$) and soil microbial biomass C/N ($P = 0.17$) were lower when exposed to E+ than E− tall fescue leaves. Because of the relatively low C/N of tall fescue leaves (13 g g^{-1}), mineralizable C/N of both leaf-amended treatments was low (11 ± 5 g g^{-1}), suggesting an abundance of available N to meet the high demands of an enlarging microbial biomass pool. The relatively high C/N of microbial biomass (i.e., 19 to 58) may have been a methodological artifact caused by immobilization of N during incubation following fumigation (Franzluebbers et al., 1994a). It is unclear whether these differences in C/N of microbial pools would be of fundamental significance to the dynamics of soil organic matter in the long term. Further studies are needed.

The fraction of inorganic N as nitrate during incubation was greatly affected by addition of tall fescue leaves compared to unamended soil (Fig. 3). For whatever reason, nitrification was either initially slower than ammonification or inhibited during the intensive release of CO_2 (or organic byproducts) during initial decomposition. By the end of 32 d of incubation, nitrification appeared to have recovered, resulting in fractions of inorganic N as nitrate nearly similar to initial values. Whether leaf tissue was E+ or E− did not have an impact on the fraction of inorganic N as nitrate (Table 4).

Active Soil Carbon and Nitrogen Exposed to Long-Term History of Endophyte Infection

There were only a few significant differences in soil C and N pools between soil histories with E− and E+ tall fescue pasture. Cumulative C mineralization on a mass of soil basis was not different between soil histories (Table 4), but per unit of soil organic C (SOC) was greater ($P = 0.08$) in E− than in E+ soil (55 vs. 52 mg CO_2–C g^{-1} SOC). Net N mineralization per g of soil (Table 4) and per unit of total soil N (TSN) (data not shown) were unaffected by soil history. Neither soil microbial biomass C (52 mg g^{-1} SOC) nor N (60 mg g^{-1} TSN) per unit of total C and N and per g of soil (Table 4) were affected by soil history of tall fescue endophyte infection. The negative effect of long-term exposure to E+ on C mineralization, but not on N mineralization and soil microbial biomass C and N, although curious, was consistent with a previous observation from this experimental site (Franzluebbers et al., 1999).

Long-term history of E+ tall fescue pasture also had an impact on the fraction of inorganic N as nitrate (Table 4). Soil exposed to E+ tall fescue pastures had

Table 4. Soil properties averaged across sampling events as affected by long-term history (i.e., 10 yr) of soil exposed to tall fescue (E− is endophyte-free, E+ is endophyte infected) and short-term addition of tall fescue leaves during 32 d of incubation. Note: Soil microbial biomass C and N were evaluated at the end of 32 d only.

	Soil history			Leaf addition			
Soil property	E−	E+	LSD (0.10)	None	E−	E+	LSD(0.10)
Potential C mineralization, μg g^{-1}	468	464	12	49	688	660	15†
Inorganic N, μg g^{-1}	47	49	8	16	59	70	10†
Mineralizable C/N, g g^{-1}	8	9	2	3	13	10	2†
Fraction of inorganic N as nitrate, g g^{-1}	0.72	0.68	0.03†	0.96	0.57	0.56	0.04†
Soil microbial biomass C, μg g^{-1}	432	475	74	291	583	487	90†
Soil microbial biomass N, μg g^{-1}	32	21	17	5	19	56	21†
Microbial biomass C/N, g g^{-1}	38	39	18	58	39	19	22 †
Specific respiratory activity, μg g^{-1} d^{-1}	23	21	5	10	25	30	6†
Ergot alkaloids in soil sediment, μg $vessel^{-1}$	1.2	2.8	1.0†	1.4	2.0	2.6	1.3
Ergot alkaloids in coarse fraction, μg $vessel^{-1}$	0.22	0.58	0.08†	0.30	0.39	0.52	0.09†
Ergot alkaloids in water extract, μg $vessel^{-1}$	0.022	0.027	0.003†	0.019	0.028	0.027	0.004†

† Significant at the $P \leq 0.10$.

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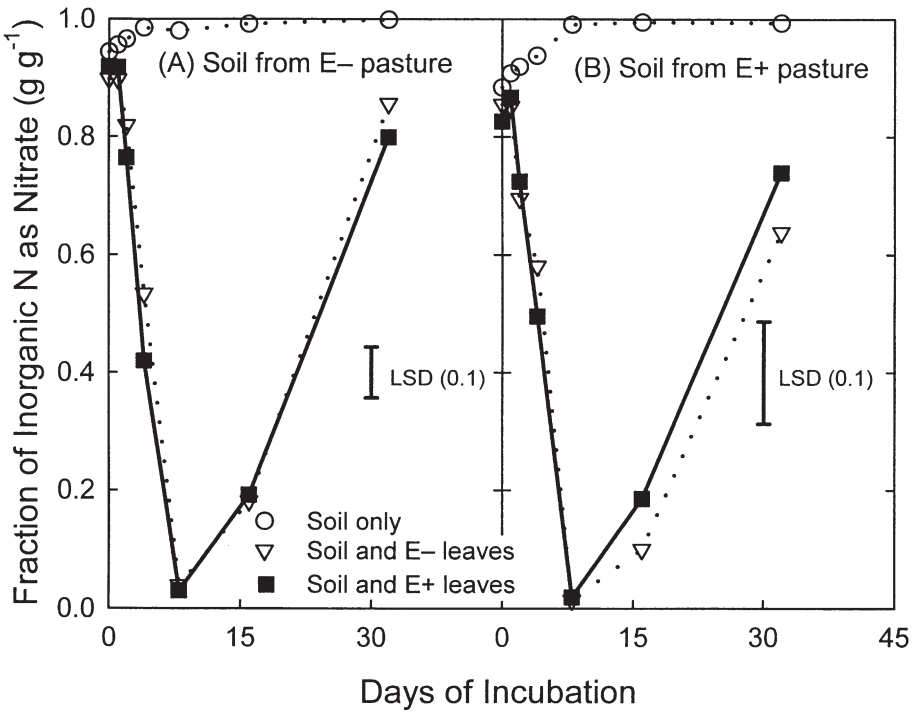

Fig. 3. Fraction of inorganic N as nitrate during 32 d of incubation as affected by addition of endophyte-free (E−) and endophyte-infected (E+) tall fescue leaf tissue in soil exposed to long-term history of endophyte-free and endophyte-infected tall fescue.

a lower fraction of inorganic N as nitrate than soil exposed to E− tall fescue pastures, suggesting that nitrification was reduced by some unknown factor in the endophyte association. Greater excretion of phenolics by E+ than E− tall fescue roots has been demonstrated (Malinowski et al., 1998). Phenolic compounds have been shown to reduce nitrification in soil (Olson and Reiners, 1983), suggesting that E+ tall fescue pastures could help conserve N in surface soil by inhibiting the formation of nitrate, which would be more susceptible to loss through denitrification and leaching.

Ergot Alkaloids

Ergot alkaloid concentration of tall fescue leaves from E+ pastures was about four times greater than from E− pastures (Table 1). The significant ergot alkaloid concentration with E− leaf addition suggests minor contamination of pastures with E+ plants. Endophyte infection frequency was <1% in E− pastures 3 yr before this experiment (Franzluebbers et al., 1999). Since leaves were collected at a time of peak ergot alkaloid concentration (i.e., late May), just a few E+ plants included in the sampling could have led to significant ergot alkaloid concentration in the bulk sample collected.

Ergot alkaloid concentration in soil sediment (<1 mm) was not significantly affected by sampling event or leaf addition (Table 2, Fig. 4). When averaged across sampling events and leaf addition treatments, soil exposed to long-term history of E+ tall fescue pasture had greater ($P = 0.01$) ergot alkaloid concentration in soil sediment than soil exposed to E− tall fescue pastures (Table 4). The presence of significantly greater ergot alkaloid concentration in soil sediment under long-term history of E+ tall fescue pasture suggests that these compounds known to cause animal health disorders remain prevalent in the soil matrix for an extended time. The consequence of this finding may have environmental implications beyond those previously described for grazing animals, and may extend toward a mechanism for altered soil microbial activity and subsequent soil organic matter accumulation. It is not clear whether ergot alkaloids found in soil were derived from ungrazed plant material or from partially digested animal byproducts, most notable urine (Stuedemann et al., 1998). Clearly, more research is needed to establish whether a functional linkage is occurring between ergot alkaloids in soil and soil organic matter dynamics.

Ergot alkaloid concentration in the coarse fraction (>1 mm, i.e., coarse sand + remaining leaves) was significantly affected by sampling event, leaf addition, and soil history (Table 2, Fig. 5). Long-term exposure of soil to E+ tall fescue pasture resulted in greater ($P < 0.001$) ergot alkaloid concentration in the coarse fraction than exposure of soil to E− tall fescue pasture (Table 4). Curiously, this difference (0.36 ± 0.04 μg vessel^{-1}) was consistent whether soil remained unamended or amended with E− or E+ tall fescue leaves, suggesting that soil history had a greater impact on the presence of ergot alkaloids than short-term E− or E+ leaf additions.

Consistent with a proxy for decomposition of ergot alkaloids in leaf tissue, the concentration of ergot alkaloids in the coarse fraction from E+ tall fescue leaves added to E− soil declined in a nonlinear manner with time (Fig. 5). This contrasted with a relatively steady concentration across sampling events when unamended and with E− leaf addition in soil from long-term E−

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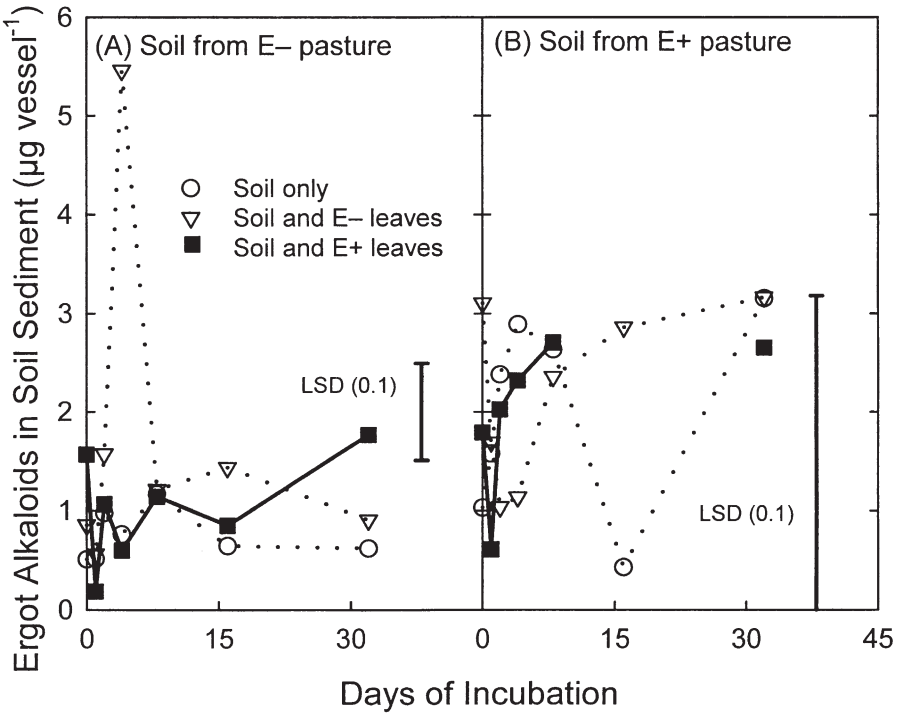

Fig. 4. Ergot alkaloids in soil sediment (<1 mm, i.e., fine sand + silt) during 32 d of incubation as affected by addition of endophyte-free (E−) and endophyte-infected (E+) tall fescue leaf tissue in soil exposed to long-term history of E− and E+ tall fescue.

pasture. The exponential decay model fitted to data had a sill of 0.14 μg vessel^{-1}, which was similar to the average concentration of control samples (0.16 μg vessel^{-1}). The size of the decomposing ergot alkaloid pool from the model (r^2 = 0.89) was 0.58 μg vessel^{-1}, with a nonlinear decay rate of 0.36 d^{-1}.

In the soil from long-term E+ tall fescue pasture, a clear indication of declining ergot alkaloid concentration in the coarse fraction was masked by large variability. The exponential decay model fitted to ergot alkaloids in the coarse fraction from E+ leaves added to soil from long-term E+ tall fescue pasture had parame-

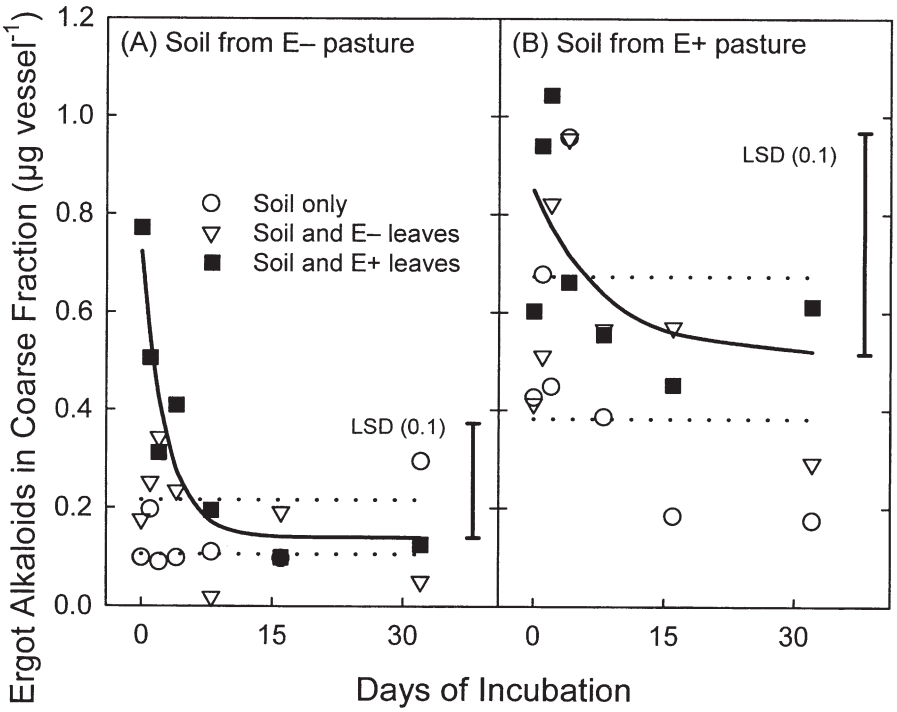

Fig. 5. Ergot alkaloids in the coarse fraction (>1 mm, i.e., coarse sand + remaining leaves) during 32 d of incubation as affected by addition of endophyte-free (E−) and endophyte-infected (E+) tall fescue leaf tissue in soil exposed to long-term history of E− and E+ tall fescue. Curve in each panel represents an exponential decay model fitted to the data for soil plus E+ leaves. Area between dotted lines represents the 95% confidence interval for the combination of soil only and soil plus E− leaves.

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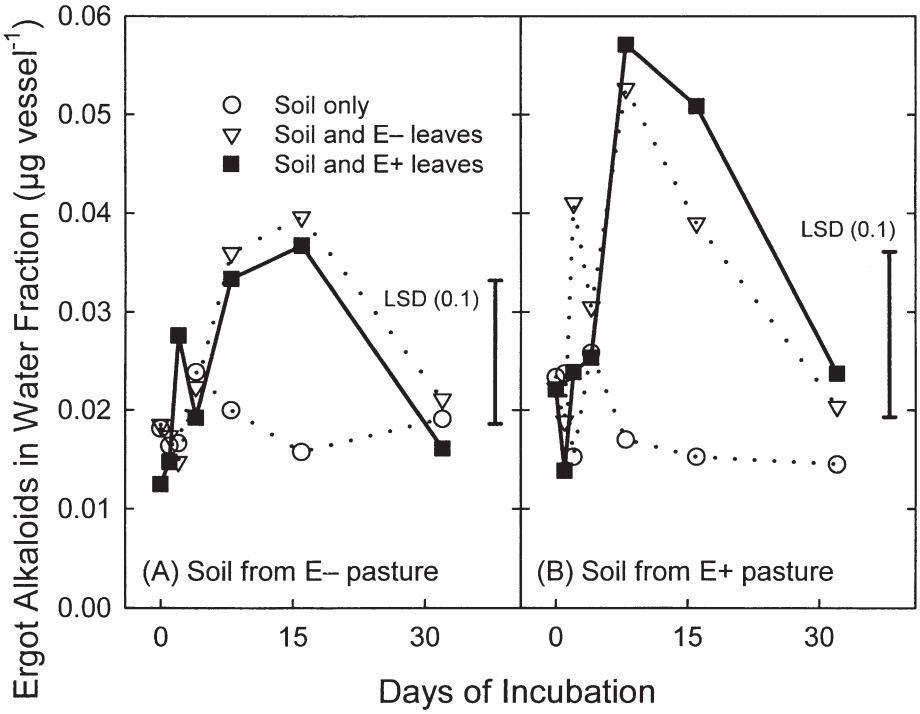

Fig. 6. Ergot alkaloids extracted in water during 32 d of incubation as affected by addition of endophyte-free (E−) and endophyte-infected (E+) tall fescue leaf tissue in soil exposed to long-term history of E− and E+ tall fescue.

ters of the following: sill = 0.52 μg vessel^{-1}, size of the decomposing ergot alkaloid pool = 0.33 μg vessel^{-1}, nonlinear decay rate of 0.13 d^{-1}, and $r^2 = 0.35$. Comparing these parameters with those obtained in long-term E− soil suggest that the presence of higher background ergot alkaloids in the coarse fraction of long-term E+ soil may have slowed decomposition of freshly added ergot alkaloids. Averaged across sampling events and soil histories, ergot alkaloid concentration in the coarse fraction was greater ($P = 0.02$) with E+ than with E− leaf addition (Table 4).

Ergot alkaloids extracted in water were near the detection limit for the enzyme-linked immonosorbance assay (1 μg L^{-1}; 0.02 μg vessel^{-1}). Although ergot alkaloid concentration in the water extract was greater in amended than unamended soils, there was no difference between E− and E+ amendment (Table 4). A small, but consistent pool of ergot alkaloids may have been water-soluble irrespective of endophyte infection. The peak in ergot alkaloid concentration at 8 and 16 d of incubation (Fig. 6) may have been due to soluble alkaloids released either from soil or decomposing leaves during a period of intense decomposition.

Ergot alkaloid concentration in soil sediment was 5.3 ± 2.3 times greater than in coarse fraction, which was 15.8 ± 7.2 times greater than in water extract (mean ± SD among soil history × leaf addition combinations).

CONCLUSIONS

Mineralizable C and soil microbial biomass C were inhibited by E+ compared with E− tall fescue leaves during a 32-d incubation. In contrast, mineralizable N and soil microbial biomass N were stimulated by E+ compared with E− leaves. Depending on the response variable, the magnitude of inhibition of C or stimulation of N was 4 to 79%. In addition, potential C mineralization as a fraction of total organic C was reduced by 5% in soil exposed to long-term E+ compared with E− pastures; potential N mineralization was not. There was no significant interaction in soil C and N transformations between long-term soil history and short-term leaf addition, suggesting that prior exposure of soil to endophyte metabolites did not alter the ability of soil to transform C and N. Ergot alkaloids, which elicit toxic symptoms in animals grazing wild-type E+ tall fescue, were found in soil with a 10-yr history of E+ tall fescue at a concentration of 28 ηg g^{-1} soil, which was 2.7 ± 1.4 times greater (mean ± SD among leaf amendments) ($P = 0.01$) than soil exposed to E− tall fescue. Addition of E+ leaves to soil resulted in a nonlinear decay rate for ergot alkaloids of 0.36 and 0.13 d^{-1} in soil with a history of E− and E+ tall fescue, respectively, suggesting that decomposition of ergot alkaloids was slower with previous long-term exposure of soil to E+ pasture. Whether ergot alkaloids in E+ tall fescue leaves have a direct or indirect effect on soil organic matter dynamics remains unknown, but the results of this study do suggest that soil biochemical transformations are altered by the presence of E+ tall fescue leaves decomposing in soil.

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